# Anti-p21 Antibody [PSH03-93] - BSA and Azide free

# HA750918

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|---|---|
| **Product Type:** | Recombinant Rabbit monoclonal IgG, primary antibodies |
| **Species reactivity:** | Mouse |
| **Applications:** | WB, IF-Cell, IHC-P, FC, IF-Tissue |
| **Molecular Wt:** | Predicted band size: 18 kDa |
| **Clone number:** | PSH03-93 |

**Description:** p21 is a potent cyclin-dependent kinase inhibitor (CKI). The p21 (CIP1/WAF1) protein binds to and inhibits the activity of cyclin-CDK2, -CDK1, and -CDK4/6 complexes, and thus functions as a regulator of cell cycle progression at G1 and S phase. The binding of p21 to CDK complexes occurs through p21's N-terminal domain, which is homologous to the other CIP/KIP CDK inhibitors p27 and p57. Specifically it contains a Cy1 motif in the N-terminal half, and weaker Cy2 motif in the C-terminal domain that allow it to bind CDK in a region that blocks its ability to complex with cyclins and thus prevent CDK activation. Experiments looking at CDK2 activity within single cells have also shown p21 to be responsible for a bifurcation in CDK2 activity following mitosis, cells with high p21 enter a G0/quiescent state, whilst those with low p21 continue to proliferate. Follow up work, found evidence that this bistability is underpinned by double negative feedback between p21 and CDK2, where CDK2 inhibits p21 activity via ubiquitin ligase activity.

**Immunogen:** Recombinant protein within mouse p21

**Positive control:** NIH/3T3 cell lysate, RAW264.7 cell lysate, RAW264.7, mouse testis tissue.

**Subcellular location:** Cytoplasm, Nucleus.

**Database links:** SwissProt: P39689 Mouse

**Recommended Dilutions:**

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| **WB** | 1:1,000 |
| **IF-Cell** | 1:100 |
| **IHC-P** | 1:200 |
| **FC** | 1:1,000 |
| **IF-Tissue** | 1:200 |

**Storage Buffer:** 1*PBS (pH7.4).

**Storage Instruction:** Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

**Purity:** Protein A affinity purified.

## Images

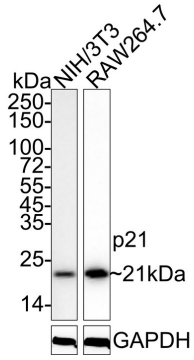


**Fig1:** Western blot analysis of p21 on different lysates with Rabbit anti-p21 antibody (HA750918) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: RAW264.7 cell lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 18 kDa
Observed band size: 21 kDa

Exposure time: Lane 1: 1 minute; Lane 2: 9 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750918) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

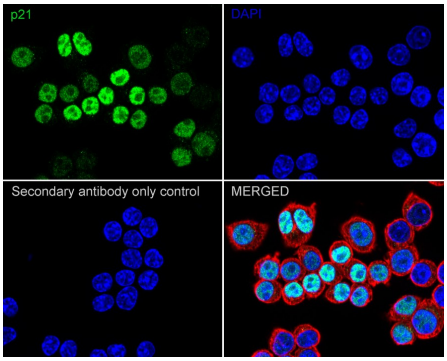


**Fig2:** Immunocytochemistry analysis of RAW264.7 cells labeling p21 with Rabbit anti-p21 antibody (HA750918) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-p21 antibody (HA750918) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

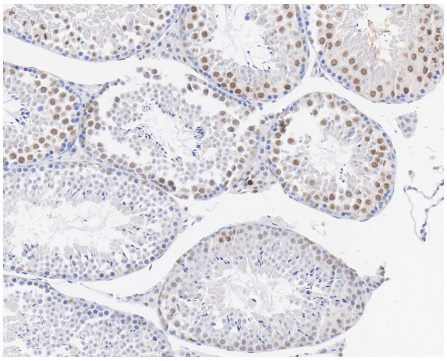

**Fig3:** Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-p21 antibody (HA750918) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with dd$H_2O$ and PBS, and then probed with the primary antibody (HA750918) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

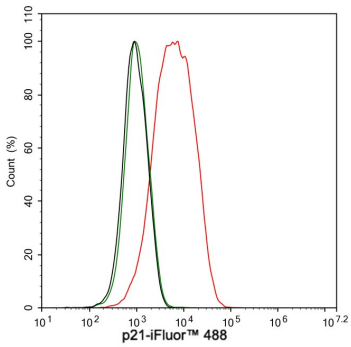


**Fig4:** Flow cytometric analysis of RAW264.7 cells labeling p21.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750918, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

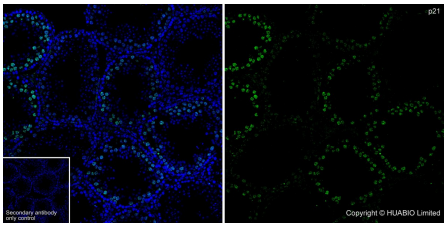


**Fig5:** Application: Immunofluorescence (IF-tissue)

Species: Mouse
Tissue: Testis
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95℃.

Wash buffer: 1× PBS
Endogenous peroxidase blocking: 3% H O , 10 minutes.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA750918, 1/200, overnight at 4℃.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 1.5 hours at room temperature.



**Background References**